Technical Manual

Total Sulfhydryl Group/Total Thiol (-SH) Colorimetric Assay Kit

- Catalogue Code: MAES0167
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

9.91-1000 μmol/L

Sensitivity:

9.91 μmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Total sulfhydryl group/total thiol (-SH) is an important protein structure and REDOX reactive group in organisms. Sulfhydryl is one of the most active and ubiquitous ligands in biological systems. It is found in most proteins, but also in some low molecular weight substances. Sulfhydryl groups play an important role in biochemical processes, not only for the REDOX mechanism, but also for the enhancement of the function of some hydrolyzed biocatalysts. Compounds containing sulfhydryl groups are called thiols. Decreased thiol levels are found in various diseases.

3. Intended Use

This kit can measure total (-SH) content in serum, plasma, animal tissue samples.

4. Detection Principle

Sulfhydryl compounds react with 5,5' -dithiobis (2-nitrobenzoic acid) under neutral or alkaline conditions to produce a yellow product which have a maximum absorption peak at 412 nm. Measure the OD value and calculate the total mercapto content indirectly.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	20 mL× 1 vial	2-8°C, 6 months
Chromogenic Agent	1.3 mL× 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard Lyophilized	Lyophilized × 2 vials	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (410-420 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Absolute ethyl alcohol

6. Assay Notes:

1. Please carry out this assay in a fume hood.

2. When the protein concentration of the sample is too high, the reaction system will become turbid after the addition of the chromogenic agent. So the sample should be diluted and tested again.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **standard solution (5 mmol/L):** Dissolve a vial of standard lyophilized with 10 mL of normal saline and mix fully. The prepared solution can be stored at 2-8°C for a day.

8. Sample Preparation

1. Serum sample:
Fresh blood was collected and placed at 25°C for 30 min to clot the blood. Centrifuge the sample at 4°C for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection.

2. Plasma sample:
The fresh blood was added into the test tube containing anticoagulant (Heparin is recommended) and mixed upside down. Centrifuge the sample at 4°C for 10 min at 700~1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection.

3. Tissue sample:
Weigh the tissue accurately and add normal saline at a ratio of weight (g): volume (mL) =1: 9, homogenize the tissue in ice bath, centrifuge at 10000 g for 10 min at 4℃, then take the supernatant for measurement.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (9.91-1000 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor
Human plasma	4-6
Human serum	4-6
Human urine	1
10% Rat liver tissue homogenate	4-6
Rabbit serum	3-5
Porcine serum	4-6
10% Rat kidney tissue homogenate	4-6
10% Rat spleen tissue homogenate	4-6

Note: The diluent is normal saline (0.9% NaCl);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 412 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

10. Operation Steps

The preparation of standard curve

Dilute standard solution (5 mmol/L) with normal saline to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1 mmol/L.

The measurement of samples

1. **Standard well:** add 40 μL of standard solution with different concentrations to the corresponding wells.
   Sample well: add 40 μL of sample to the corresponding wells.
   Control well: add 40 μL of sample to the corresponding wells.

2. Add 150 μL of buffer solution to each well.

3. Add 10 μL of chromogenic agent to standard wells and sample wells.

4. Add 10 μL of absolute ethyl alcohol (self-prepared) to control wells.

5. Mix fully and stand for 10 min at room temperature. Then measure the OD value of each well at 412 nm with microplate reader.

Operation Table

	Standard well	Sample well	Control well
Standard solution with different concentrations (μL)	40		
Sample (μL)		40	40
Buffer solution (μL)	150	150	150
Chromogenic agent (μL)	10	10	
Absolute ethyl alcohol (self-prepared) (μL)			10
Mix fully and stand for 10 min at room temperature. Then measure the OD value of each well at 412 nm with microplate reader.			

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) sample:

Total (-SH) content (μmol/L)
$= (\Delta A_{412} - b) \div a \times 1000^* \times f$

2. Tissue sample:

Total (-SH) content (μmol/g fresh weight)
$= (\Delta A_{412} - b) \div a \div (m \div V) \times f$

y: $OD_{Standard} - OD_{Blank}$. ($OD_{Blank}$ is the OD value when the standard concentration is 0)
x: The concentration of standard
a: The slope of standard curve
b: The intercept of standard curve
f: Dilution factor of sample before test
ΔA_{412}: $OD_{Sample} - OD_{Control}$
1000*: 1 mmol/L=1000 μmol/L
m: The fresh weight of sample, g
V: The volume of normal saline in preparation step of tissue sample, mL

12. Performance Characteristics

Detection Range	9.91-1000 μmol/L
Sensitivity	9.91 μmol/L
Average recovery rate (%)	104
Average inter-assay CV (%)	2.9
Average intra-assay CV (%)	2.5

Analysis

For human serum, take 40 μL of human serum sample, dilute with normal saline for 5 times, carry the assay according to the operation table.

The results are as follows:
Standard curve: y = 1.5351 x - 0.0023, the average OD value of the sample is 0.216, the average OD value of the control is 0.115, and the calculation result is:

Total (-SH) content (μmol/L) = (0.216 – 0.115 + 0.0023) ÷ 1.5351 ×1000 × 5
= 336.46 μmol/L

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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